BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1. An endogenous RNAi

The sequence of a human let-7 RNA gene is composed of a line of nucleotides. The blue one stands for the sequence encoding the sense strand of let-7 RNA, while the red is for the antisense strand of let-7 RNA. The green one is related to the change of nucleotides in let-7 RNA gene.

AL158152.18 GI:15212042, Human DNA sequence from clone RP11-2B6 on chromosome 9q22.2-31.1

```
37801 tcacacagga aaccaggatt accgaggagg aaaaaaagcc ttcctgtggt gctcaactgt
37861 gattcctttt caccattcac cctggatgtt ctcttcactg tgggatgagg tagtaggttg
37921 tatagtttta gggtcacacc caccactggg agataactat acaatctact gtctttccta
37981 acgtgataga aaagtctgca tccaggcggt ctgatagaaa gtcagttaac taattgtaca

38221 gataatttta tgttgaaatt ttctttcgaa agagattgta ctttccattc cagaagaaaa
38281 cattgctcta tcagagtgag gtagtagatt gtatagttgt ggggtagtga tttttaccctg
38341 ttcaggagat aactatacaa tctattgcct tccctgagga gtagacttgc tgcattattt
38401 tctttttatt tagatgatat taaaactcag aagaattaat tttgacattt tgtatttaca

40681 aattagaaac aaaactcaaa gaacatgacc taatttaaca ggttaatttg aagtgcatct
40741 gccaagtaga agaccagcaa gaaaaaaaaa atgggttcct aggaagaggt agtaggttgc
40801 atagttttag ggcagggatt ttgcccacaa ggaggtaact ataccacctg ctgcctttct
40861 tagggcctta ttattcaccg ataacctgtt tccttgctac tttgctttgg tgtaagcaga
```

Fig 2. BLAST Multiple Sequence Alignments:

A set of sequences was fished out by a query sequence of human insulin-like growth factor 2 gene.

Total 66 pages
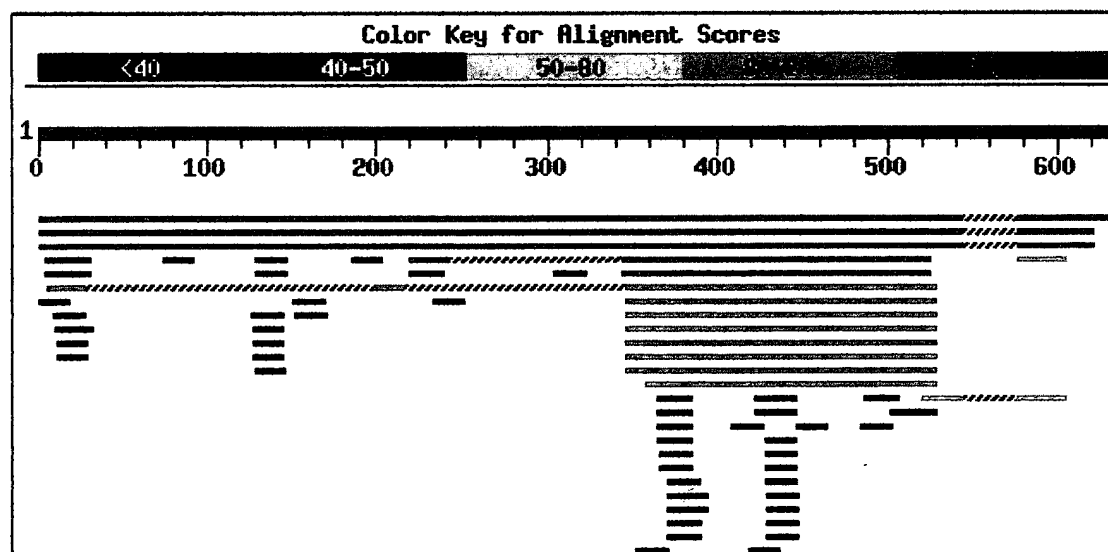
Score   E

Sequences producing significant alignments: (bits) Value

```
gi|32997|emb|X07867.1|HSIGF24B    Human DNA for insulin-like g...   1009   0.0
gi|33003|emb|X03562.1|HSIGF2G     Human gene for insulin-like g...   722   0.0
gi|183100|gb|M22373.1|HUMGFIA2    Human insulin-like growth fa...    722   0.0
gi|2909374|emb|Y16533.1|OAR16533  Ovis aries IGF-II gene, ex...      222   3e-55
gi|405977|gb|U00665.1|OAINIGFII4  Ovis aries insulin-like gr...      208   4e-51
gi|2558855|gb|AF020599.1|ECILGF22 Equus caballus insulin-li...       198   4e-48
gi|2689877|gb|U71085.1|MMU71085   Mus musculus insulin-like g...     174   5e-41
gi|15208269|dbj|AP003184.1|AP003184  Mus musculus genomic DN...      174   5e-41
```

Fig 3. CLUSTAL W (1.81) Multiple Sequence Alignments:

The homologous sequences of human insulin-like growth factor 2 gene derived from different species were aligned and compared with each other by using CLUSTAL W Multiple Sequence Alignments.

Sequence format is Pearson
Sequence 1: Ymossambicus   570 bp
Sequence 2: AF79Tilapiamossamb   549 bp
Sequence 3: Y9Oreochromismossa   387 bp
Sequence 4: AF7Gallusgallus   1066 bp
Sequence 5: AJZebrafinch   564 bp
Sequence 6: MMouseinsulin-lik   543 bp
Sequence 7: Rat IGF-2   543 bp
Sequence 8: human IGF-2   543 bp
Start of Pairwise alignments

```
MMouseinsulin-lik      AGCCGT--GCCAACCGTCGC------AGCCGTGGCATCGTGGAAGAGTGCTGCTTCCGC 219
Rat                    AGCCGT--GCCAACCGTCGC------AGCCGTGGCATCGTGGAAGAGTGCTGCTTCCGC 219
human                  AGCCGT--GTGAGCCGTCGC------AGCCGTGGCATCGTTGACGAGTGCTGTTTCCGC 219
Y9Oreochromismossa     AGCACGGGTAACAACCGACGCGCCGCCAGACCCGTGCGGATCGGTAGACGGAGTGTTGTTTCCGT 66
AF7Gallusgallus        AGCACGTCTAACACCACACGCTCCCAGAACCGTGGTATCGTGGAGGAGTGTTGTTTCCGT 718
AJZebrafinch           GGACCA--AATAACCGCCGGTTC--AACCGGGGGATCGTGGACGAGTGCTGCTTTCGG 219
Ymossambicus           GGCTATGGCCCCAGTGCAAGGC--GGTCACGTGGCATCGTGGACGAGTGCTGCTTCCAA 276
AF79Tilapiamossamb     GGCTATGGCCCCAGTGCAAGGC--GGTCACGTGGCATCGTGGACGAGTGCTGCTTCCAA 276
                         *       *        *       ** ** ** ** ** ***** ** ** *

MMouseinsulin-lik      AGCTGCGACCTGGCCCTGCCTGGAGACATACTGTGCCCACCCCCGCCAAGTCCGAGAGCGAC 279
Rat                    AGCTGCGACTTGGCCCTCCTGGAGACATACTGTGCCCACCCCCGCCAAGTCCGAGAGCGAC 279
human                  AGCTGTGACCTGGCCCTCCTGGAGACGTACTGTGCCTACCCCCGCCAAGTCCGAGAGGGAC 279
Y9Oreochromismossa     AGCTGTGACCTCAAGCTACTGGAGCAGTACTGTGCCAAACCTGCCAAGTCAGAAAGGGAC 126
AF7Gallusgallus        AGCTGTGACCTCAACCTGTTGGAGCAGTACTGTGCCAAAGCTGCCAAGTCAGAGAGGGAC 778
AJZebrafinch           AGCTGTGACCTGGCTCTGCTGGAGACGTACTGCGCCAAATCCGTCAAGTCCGAGCGTGAC 279
```
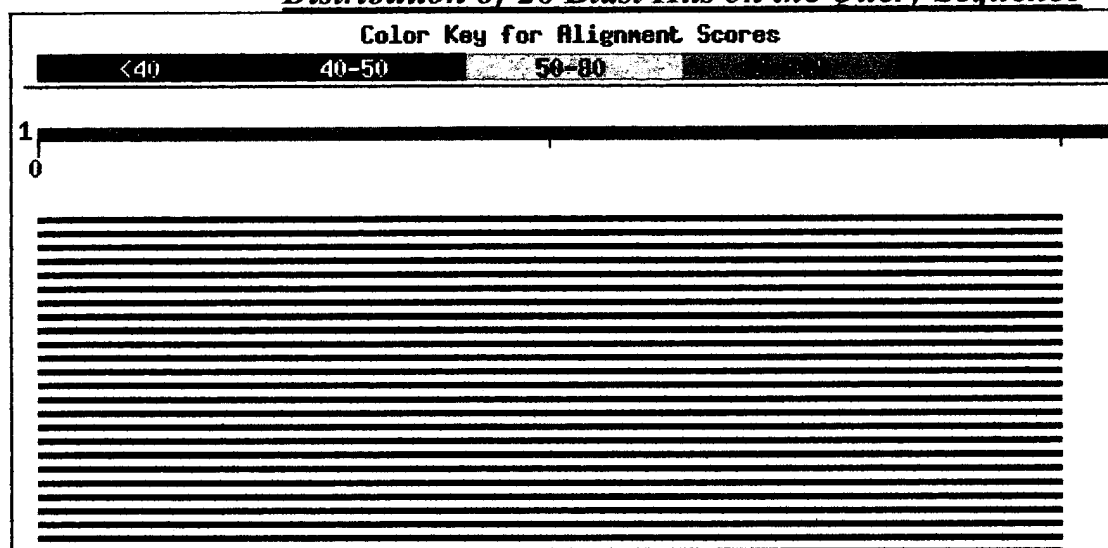
Ymossambicus	AGCTGTGAGCTGCAGCGCCTTGAGATGTACTGTGC—ACCTGTCAAGACTCCCAA-GAT 332
AF7911 apiamossamb	AGGTGTCAGCTGCAGCGCCTTGAGATGTACTGTGC—ACCTGTCAAGACTCCCAA-GAT 332
	***** ** * * * *** ***** ** * * **** * **

Fig. 4a. BLAST Search.

Database: nt   951,499 sequences; 3,985,165,516 total letters

Distribution of 26 Blast Hits on the Query Sequence


```
Score    E
Sequences producing significant alignments:                      (bits) Value
```

gi|14773163|ref|XM_006402.3| Homo sapiens insulin-like grow... 42 0.002
gi|14773161|ref|XM_028186.1| Homo sapiens insulin-like grow... 42 0.002
gi|14773159|ref|XM_028187.1| Homo sapiens insulin-like grow... 42 0.002
gi|14773157|ref|XM_028184.1| Homo sapiens insulin-like grow... 42 0.002
gi|14773155|ref|XM_028189.1| Homo sapiens insulin-like grow... 42 0.002

>gi|14773163|ref|XM_006402.3| Homo sapiens insulin-like growth factor 2 (somatomedin A)
(IGF2),   mRNA   Length = 1202

Score = 42.1 bits (21), Expect = 0.002
Identities = 21/21 (100%)
Strand = Plus / Plus

```
Query: 1    agccgtggcatcgttgaggag 21
            | | | | | | | | | | | |
Sbjct: 544  agccgtggcatcgttgaggag 564
```

The specificity of a query sequence selected by systematic selection method was evaluated by Blast

search. The results indicated that the total hits were 26, 25 of which are belong to the same gene

family, and only one of which is derived from other gene family, suggesting that this query sequence has very high specificity. The experiment indicated that the systematic selection method is a useful and good method even though the process of selection was pretty complicated.

Table 4b. gi|33003|emb|X03562.1|HSIGF2G Human gene for insulin-like growth factor II

Seq ID	Total Hit	100% Match	80-95% Match	<80% Match	Pattern	Start Point	Sequence	End Point
1	36	25n		11n	None	7534	agccgtggcatcgttgagg	7552
2	83	25n	1n	57n	None	7543	atcgttgaggagtgctgtt	7561
3	84	25n	1n	58n	None	7550	aggagtgctgtttccgcag	7568
4	65	25n		40n	None	7553	agtgctgtttccgcagctg	7571
5	42	25n	2n	15n	None	7589	agacgtactgtgctacccc	7607
6	45	25n		20n	None	7591	acgtactgtgctacccccg	7609
7	45	25n	1n	16n	None	7595	actgtgctacccccgccaa	7613
8	51	25n	1n	25n	None	7603	accccgccaagtccgaga	7621

The table 4b listed other sequences selected by the random selection method. The results showed that all the sequences were not so good as the sequence shown in the Fig.4, suggesting that the systematic selection method is superior to the random selection method.

Fig. 5. BLAST search for two sequence alignment

This method is useful for selecting homologous sequences with a big gap or different sequence between. After localizing the region of homologous sequence, interested sequence will be selected out as query sequence for further searching and comparing.


```
Sequence 1 lcl|seq_1 Length 651 (1 .. 651)
Sequence 2 lcl|seq_2 Length 649 (1 .. 649)
```
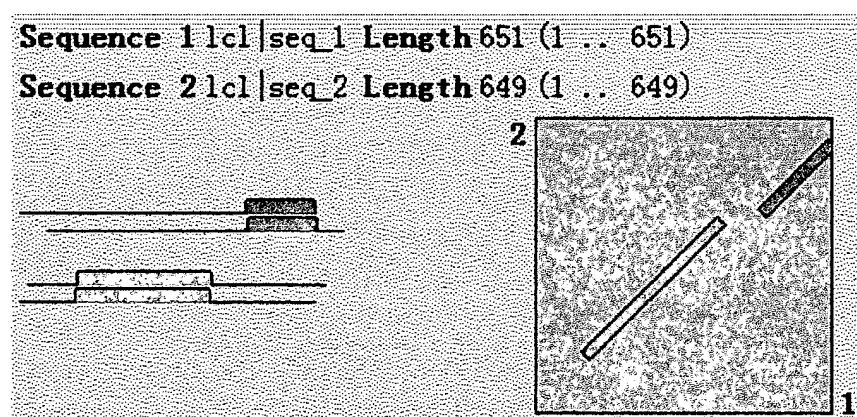
Fig. 6 BLAST search for an endogenous RNAi gene sequences from different species

Query= (21 letters)   **Database: nt**
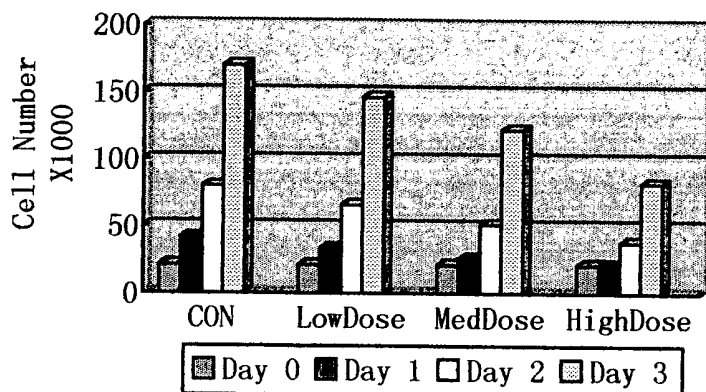
Effects of Dermogene on Melanoma Cells

Fig 10. displayed that growth-inhibitory effects of Dermogene on cultured human melanoma cells were mediated by the administration of a group of siRNAs for one time. For this, 1 ml of melanoma cell suspension in culture medium (2×10^4/ml) was placed in each well. Cell growth was evaluated on days 0, 1, 2 and 3 by an automatic counter made in Coulter Corporation ($n = 3$). Values given are means ± SD expressed as number of cells × 10^4/ml.

Fig 11. The in vitro effects of Dermogene on the survival and proliferation of human melanoma cells.

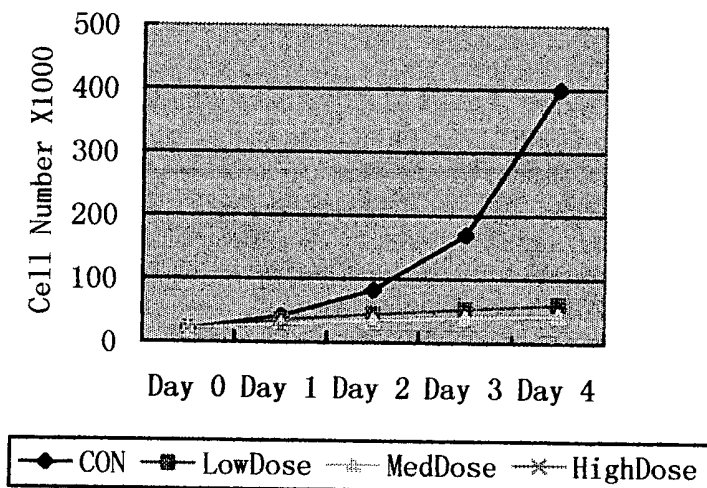Effects of Dermogene on the proliferation of melanoma cells

Fig 11. displayed that growth-inhibitory effects of Dermogene on cultured human melanoma cells were mediated by the administration of a group of SDSOs every day for four days. For this, 1 ml of

melanoma cell suspension in culture medium (2×10^4/ml) was placed in each well. Cell growth was evaluated on days 0, 1, 2, 3 and 4 by an automatic counter made in Coulter Corporation ($n = 3$). Values given are means ± SD expressed as number of cells × 10^4/ml.

Fig 12. In vivo pharmaceutical effects of Dermogene on melanoma cells.

## In Vivo Effects of siRNAs on Melanoma Cells
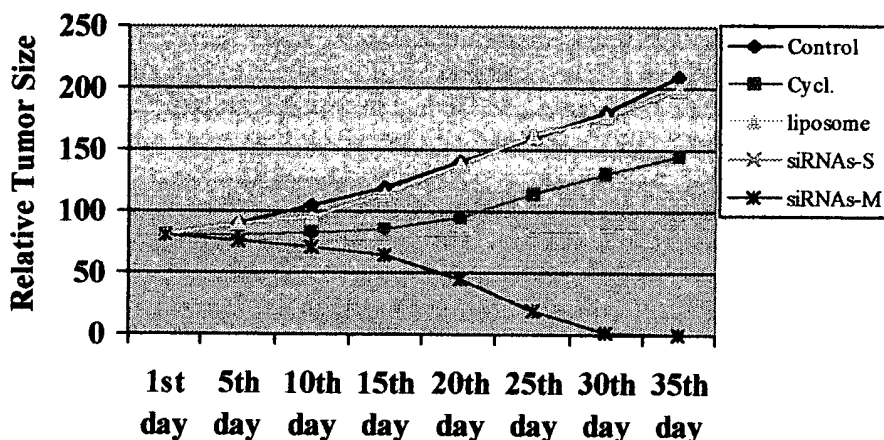
Figure 12. Effects of injection of cationic liposomes containing Dermogene on the growth of human melanoma transplanted to nude mice. The dark blue line is related to intratumoral injections of PBS (30ul) every other day. The yellow line means intratumoral injections of empty liposomes (200 nmol liposome in 30ul) every other day. The light blue line stands for intratumoral injection of liposomes containing Dermogene (5ug mixture of Dermogene and 200 nmol liposome in 30 ul) every other day. The pink line means intratumoral injection of 30 ul liposomes containing 1mg Cyclophosphamide. The dark brown line stands for intratumoral injections of liposomes containing Dermogene (5ug mixture of Dermogene and 200 nmol liposome in 30 ul) and 1mg Cyclophosphamide every day. Melanoma nodules were evaluated by measuring the size every 5 days with the aid of microcallipers, and tumor volume and relative tumor size were calculated.

Fig.13. The biological roles of Leukogene on CML cells.

Fig 13. illustrated the effects of Leukogene in the dose of 100 ng/ml and 200 nmol empty liposome on the proliferation of CML cells derived from (CML1 and CML1C) patient 1, (CML2 and CML2C) patient 2, and (CML3 and CML3C) patient 3. Cell numbers are the average obtained from three wells.

Effects of Leukogene on CML Cells
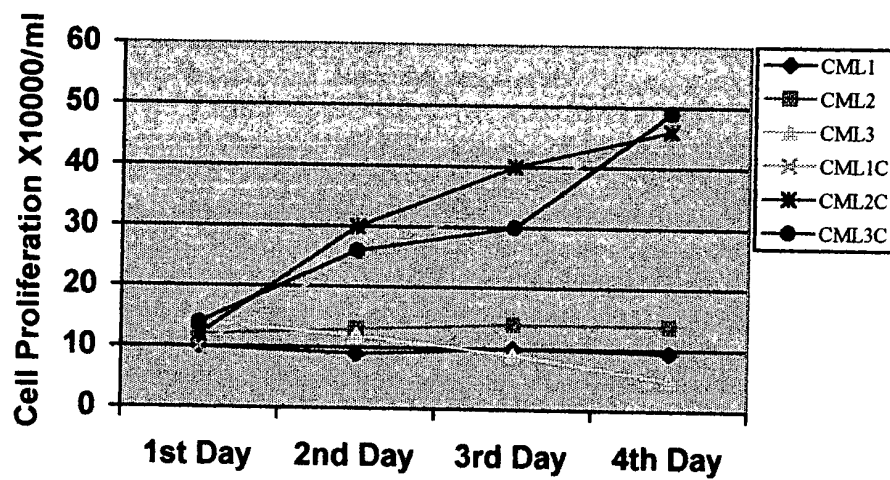
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Legal Date: 09-16-2002

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